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Increasing Positive Clonality Detection Rate in Multiple Myeloma Research Samples Using NGS Characterization of Multiple B cell Receptors in a Single Reaction

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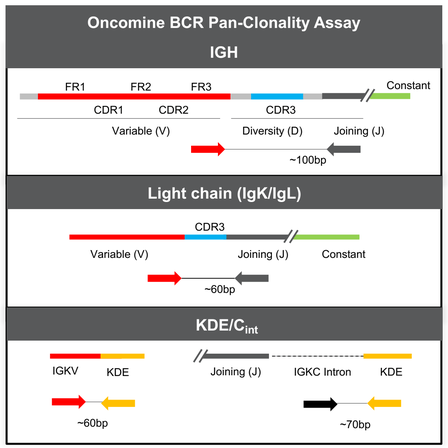
INTRODUCTION

B cell repertoire analysis by next-generation sequencing (NGS) is at the forefront of leukemia and lymphoma research. Some advantages provided by NGS-based techniques include a lower limit-of-detection and simpler paths to standardization compared to other methods. Importantly, in research of post-germinal B cell disorders, such as multiple myeloma, NGS methods allow for the study of clonal lineage based on somatic hypermutation (SHM) patterns. Current targeted NGS assays require multiple libraries to survey each B cell receptor chain (IGH, IGK, IGL), and this fact is highlighted when initial clonality detection fails due to mutations under primer binding sites. This issue can be especially true with multiple myeloma which has a high rate of SHM. **To address these issues, we have developed an assay for B cell analysis, based on Ion AmpliSeq™ technology, which enables efficient detection of IGH, IGK, and IGL chain rearrangements in a single reaction.**

PANEL DESIGN

A



B

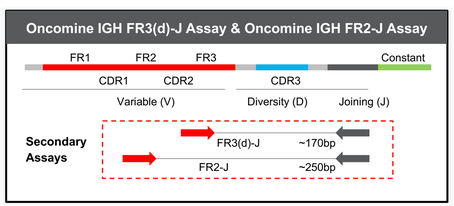


Figure 1. (A) B cell Pan-Clonality Assay (IGH/K/L) contains FR3-J primers for IGH, IGK, IGL, as well as KDE/Cint primers in a **single library preparation reaction. (B)** Schematic of two secondary assays that target the distal region of FR3 (FR3(d)-J) and FR2-J regions.

MATERIALS AND METHODS

The BCR Pan-Clonality panel targets the framework 3 (FR3) portion of the variable gene and the joining gene region of heavy- and light-chain loci (IGH, IGK, IGL) for all alleles found within the IMGT database, enabling readout of the complementary-determining region 3 (CDR3) sequence of each immunoglobulin chain. To maximize sensitivity, we included primers to amplify IGK loci rearrangements involving Kappa deletion element and the constant region intron. To evaluate assay performance, we conducted reproducibility studies and clonality assessment using gDNA from a total of 45 multiple myeloma research samples. All multiple myeloma cases examined in this work were confirmed clonal previously by light chain restriction via flow cytometry or IHC/ISH in tissue sections. Sequencing and clonality analysis was performed using the Ion GeneStudio S5 System and Ion Reporter 5.16 analysis software.

Multiple Myeloma cohort:

- 45 Clinical research BMA specimens
- **Clinically characterized**
  Wide range of disease burden by flow cytometry and/or IHC
- 16 lambda light chain positive samples
- Included known polyclonal control samples

Goal:

- Assign clonotype to respective MM research samples through detection of clonal BCR rearrangements
- **Following clonality assignment guidelines from:**
  Arcila, Maria E. et.al. Establishment of Immunoglobulin Heavy (IGH) Chain Clonality Testing by Next-Generation Sequencing for Routine Characterization of B-Cell and Plasma Cell Neoplasms. Journal of Molecular Diagnostics, Vol. 21, No. 2, March 2019.
- Achieve clonal rearrangement detection in >85% MM samples

Commercially procured control:

- **DNA from cell line(s) with IgH and IgK rearrangements**
- **Spiked into samples at 100 cell equivalents**
  - Re: Each sample contains approx. 4 million cell equivalents
  - Internal control should be detected at a ~10^{-4} frequency

RESULTS

Oncomine™ BCR Pan-Clonality Assay

Samples	Total Tested	Total positive (IGH) [%]	Total positive (IGL) [%]	Total positive (IGH+IGL) [%]
Clinical Research Samples (MM)	45	34 [76%]	41 [91%]	**42 [93%]**

Oncomine™ IGH FR3(d)-J and FR2-J Assays

Samples	Total Tested	Total positive FR3(d)-J [%]	Total positive FR2-J [%]
Clinical Research Samples (MM)	14**	7 [50%]	7 [50%]

** Testing included 11 samples not detected with the Pan-Clonality (IGH) and 3 borderline cases

Comparison of results from our MM (Post-GC) cohort (N=45) with a cohort of B-ALL (Pre-GC) samples (N=11)

Biased IGHV & Stereotypy

1. Both cohorts showed potential biased IGHV usage with **V3-11** in **5/45 MM** samples and in **5/12 B-ALL** samples.
2. **IGHV4-34** was detected in **5/45 MM** cases (and one B-ALL case).
   The high prevalence of IGHV4-34 in several lymphoma subtypes (e.g. BL, CLL, MZL) has led to a suggestion that autoreactive antigens may drive the expansion of some B-cell lymphomas.

SHM*

1. All but two MM samples have SHM while all B-ALL samples have 0% SHM.
   When sequenced with FR1-J primers - low SHM in two MM samples (0.44% and 1.3%)
2. Most MM samples have high levels of SHM (**6/35 > 10% SHM**)
   Ion Reporter lineage analysis tool we identified **8/45 MM** cases with 5 or more clones - one case with 23 clones!

*Oncomine BCR Pan-Clonality reports somatic hypermutation rate for the portion of the IGHV gene covered by the assay.

14/16 (88%)

lambda-positive MM samples were deemed clonal by the IgK/IgL assay component

A clonal lambda light chain rearrangement was identified in 10/14 (71%) of the IgK/IgL clonal samples

In two samples **only a clonal lambda rearrangement** was detected.

Frequency of commercially available control detected by BCR Pan-Clonality Assay

	Polyclonal sample	Clonal sample 1	Clonal sample 2	Clonal sample 3
BCR Pan Clonality (IgK)	0.79×10^{-4}	0.78×10^{-4}	0.83×10^{-4}	0.87×10^{-4}
BCR Pan-Clonality (IgH)	1.14×10^{-4}	3.47×10^{-4}	4.88×10^{-4}	2.35×10^{-4}

CONCLUSION

Clonality assessment of multiple myeloma clinical research samples show a **93% overall positive detection rate by an assay which combines the IGH, IgK, and IgL chains in a single reaction** using published guidelines for clonality assignment. 34 of 45 samples show positive detection of an IGH rearrangement, while 41 of 45 showed positive detection of at least one light chain receptor. In total, 42 of 45 samples were deemed clonal by the single tube assay based on detection for one or more receptor. Clonality results for this sample set are well correlated with orthogonal data from flow, IHC/ISH, or alternate NGS technologies. These results demonstrate the utility of a novel Ion AmpliSeq-assay for combined clonality analysis of B cell receptor heavy and light chains. We expect this assay to simplify workflow and open new paths for research in B cell disorders.

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ThermoFisher SCIENTIFIC

https://www.oncomine.com/immune-repertoire-sequencing